Influence of bioturbation by the polychaete Nereis diversicolor on the structure of bacterial communities in oil contaminated coastal sediments

Philippe Cuny, Gilles Miralles, Veronique Cornet, Monique Acquaviva, Georges Stora, Vincent Grossi, Franck Gilbert

Influence of bioturbation by the polychaete *Nereis diversicolor* on the structure of bacterial communities in oil contaminated coastal sediments

Philippe Cuny [a,*], Gilles Miralles [a], Véronique Cornet-Barthaux [b], Monique Acquaviva [a], Georges Stora [a], Vincent Grossi [c], Franck Gilbert [a]

[a] *Laboratoire de Microbiologie, Géochimie et Ecologie Marines, UMR CNRS 6117, Campus de Luminy, Case 901, F-13288 Marseille Cedex 9, France*
[b] *Laboratoire d'Océanographie et de Biogéochimie, UMR CNRS 6535, Campus de Luminy, Case 901, F-13288 Marseille Cedex 9, France*
[c] *Paléoenvironnements et Paléobiosphère, UMR CNRS 5125 PEPS, France, Université Claude Bernard Lyon 1, Campus de la Doua, Bâtiment Géode, F-69622 Villeurbanne cedex, France*

Abstract

Patterns of change in the structure of bacterial communities monitored by ribosomal intergenic spacer analysis (RISA) in oil contaminated sediments inhabited or not by the marine polychaete *Nereis diversicolor* were studied during 45 days under laboratory conditions. Results supported by principal component analysis showed a marked response of the bacterial communities to the oil contamination and to the presence of *N. diversicolor*. Phylogenetic affiliation of specific RISA bands showed that, in the contaminated sediments, the presence of the marine polychaetes favoured the development of bacteria which may play an active role in natural bioremediation processes of oil polluted environments.

Keywords: Bacterial communities; Bioturbation; Oil spills; Marine sediments; Bioremediation; *Nereis diversicolor*

1. Introduction

Marine sediments, particularly those in industrial areas, are frequently polluted with petroleum hydrocarbons as the results of accidental spills, industrial and urban runoff and shipping activities (Head and Swannell, 1999). Bioturbation processes due to natural macrobenthic communities can have a significant influence on the qualitative and quantitative fate of hydrocarbons following oil contamination (Grossi et al., 2002). Sediment reworking can, for instance, alter oxic/anoxic boundaries, affect microbial populations directly or indirectly, cause vertical transport of particles and lead to movements of particles across oxic and anoxic boundaries (Aller, 1994). The frequency and duration of these processes may notably modulate the degradation rates of hydrocarbons (Granberg et al., 2005). Macrobenthic animals, especially sediment-dwelling polychaetes, are known to affect the release and distribution of polycyclic aromatic hydrocarbons (PAHs) associated with the sediment through their bioturbating activities. For example, *Arenicola marina* and *Nereis diversicolor* were shown to significantly enhance the fluxes of sediment-associated pyrene and metabolites into overlying water (Christensen et al., 2002b). In the same way, the polychaetes *Nereis virens* and *Capitella capitata* are known to enhance microbial degradation of PAHs such as benzo[a]anthracene (Gardner et al., 1979; McElroy et al., 1990) and anthracene (Bauer et al., 1988), respectively. In addition to displacing hydrocarbons from oxic to anoxic layers, macrobenthic organisms can also act on hydrocarbon fate by producing digestive solubilizers which enhance the bioavailability of

* Corresponding author. Tel.: +33 4918 29147; fax: +33 4918 29641.
E-mail address: philippe.cuny@univmed.fr (P. Cuny).

doi:10.1016/j.marpolbul.2006.12.008

hydrocarbons to hydrocarbon degrading bacteria (i.e., hydrocarbonoclastic bacteria), and may increase their exportation into the water column. Digestive fluids of deposit feeders have been shown to be far more effective than seawater in solubilizing PAHs (Penry and Weston, 1998; Weston and Mayer, 1998) and other sediment-bound organic contaminants (Ahrens et al., 2001). For instance, biosurfactants are present in high amounts in the digestive tract of benthic macrofauna (Ahrens et al., 2001) which ingests the surface sediment several times a year (Myers, 1977). Consequently, sediment-bound hydrocarbons are solubilized during the gut transit of the organisms (Gilbert et al., 2001). The solubilization by benthic-surfactants has a positive effect on biodegradation, which depends on the dispersion state of hydrocarbons (Bertrand et al., 1993; Bonin and Bertrand, 1999).

In addition, previous studies have shown that *N. virens* burrow sediments harbour PAH-degrading bacteria and that degradation rates for added PAHs are higher in burrowed sediments than in non-burrowed ones (Chung and King, 1999). In addition, a new species of hydrocarbonoclastic bacteria, *Lutibacterium anuloederans*, has been isolated from *N. virens* burrow walls by using enrichment cultures on phenanthrene (Chung and King, 2001).

Bioturbation also influences the vertical distribution and the composition of sedimentary bacterial communities (Dobbs and Guckert, 1988; Findlay et al., 1990; Grossmann and Reichardt, 1991; Goñi-Urriza et al., 1999; Wilde and Plante, 2002; Papaspyrou et al., 2004; Plante and Wilde, 2004; Grossi et al., 2006; Papaspyrou et al., 2006). Unfortunately no information is currently available on the specific effects of bioturbation on the structure of bacterial communities in oil contaminated sediments. Yet the selection of specific bacterial groups due to bioturbation activities may be a key factor in controlling the fate of hydrocarbons within sediments. To clarify this point, the patterns of change in the structure of bacterial communities monitored by ribosomal intergenic spacer analysis (RISA) were studied in oil contaminated sedimentary microcosms inhabited or not by the polychaete *N. diversicolor*.

2. Material and methods

2.1. Microcosm experiment

Sediments and *N. diversicolor* individuals were collected in Carteau cove (Gulf of Fos, France, Mediterranean Sea). The sediment organic matter content, determined by loss of ignition at 550 °C after 24 h, was $1.33 \pm 0.03\%$. Worms were acclimated for 6 days at in situ temperature (15 ± 1 °C) in uncontaminated sediment overlaid with 28‰ seawater, before being transferred to sediment microcosms.

Twelve sediment microcosms were set up in 2.5 l aquarium (11 cm wide, 19 cm long and 12 cm high) filled with sieved (1 mm mesh) and homogenized sediments to obtain a 9-cm high sedimentary column. Half of the sediments were previously contaminated with BAL-250 oil (Blend Arabian Light oil topped at 250 °C) to a concentration of 7 g kg^{-1} wet sediment. Ten individuals of the polychaete *N. diversicolor* (12 ± 1 cm long; mean $\pm$ SD, $n = 60$) were then introduced into each of three control (uncontaminated) and three contaminated microcosms to reach a density of 500 polychaetes m^{-2}.

Four different sediment microcosms were considered: uncontaminated sediment without worms (CTRL), uncontaminated sediment with *N. diversicolor* (CTRL + Nd), contaminated sediment without worms (OIL) and contaminated sediment with *N. diversicolor* (OIL + Nd). Microcosms were left to incubate at 15 ± 1 °C during 45 days. Ambient air was pumped continuously through the water column. At the end of the experiment the worms were removed and the sediments were homogenized by mechanical stirring before sampling.

2.2. DNA extraction

The protocol for DNA extraction was modified from Tsai and Olson (1991). Freeze-dried sediments were carefully ground, and subsamples (0.25 g dry weight) of each microcosm were mixed with 500 µl of TES buffer (100 mM Tris–HCl (pH = 8.0), 100 mM Na_2HPO_4 (pH = 8.0), 100 mM Na_2EDTA (pH = 8.0), 1.5 M NaCl). Three cycles of freezing (liquid nitrogen, 2 min) and thawing (water bath 100 °C, 2 min) were conducted to allow the lysis of microbial cells (Roose-Amsaleg et al., 2001). Lysozyme (10 mg, Sigma) and proteinase K (7 µl of a 10 mg ml^{-1} solution, Boehringer Mannheim) were then added to the samples which were incubated for 2 h at 37 °C under agitation (300 rpm). Following the addition of hexadecyltrimethylammonium bromide (10 mg, Sigma), the samples were further incubated for 2 h at 65 °C under agitation (300 rpm) before centrifugation at 12,000*g* (20 °C, 15 min). Supernatants were mixed with an equal volume of chloroform-isoamyl alcohol (24:1, v/v). The aqueous phase was recovered by centrifugation (12,000*g*, 20 °C, 15 min) and precipitated with isopropanol overnight at room temperature. The crude DNA pellets were obtained by centrifugation at 15,000*g* (room temperature, 30 min.) and were gently washed with 70% ethanol (−20 °C). Ethanol was removed after centrifugation (15,000*g*, 20 °C, 15 min.) and air-drying (10–15 min). The pellets were suspended in 50 µl of sterile ultrapure water and stored at −20 °C.

2.3. RISA

Ribosomal intergenic spacer analysis (RISA) was used to characterize changes in the genetic structure of bacterial communities (Borneman and Triplett, 1997; Kirk et al., 2004; Dorigo et al., 2005). The intergenic transcribed spacer (ITS) region between the large and the small subunit of ribosomal sequences was amplified by PCR using 5 ng of purified template DNA with the primers FGPS1490-72 and

FGPL132-38 (Normand et al., 1996). Amplification reactions were performed in a final volume of 50 μl containing HighFidelity buffer (5 μl of 10 × dilution), $MgCl_2$ (2.5 mM), dNTP (200 μM of each), primers (25 pmol of each), T4 gene 32 protein (1 μg, Boehringer Mannheim), and TripleMaster polymerase (1.75 units, Eppendorf AG). Reactions were performed in an automated DNA thermal cycler (Eppendorf Mastercycler) for 25 cycles. After an initial denaturing step (30 s at 94 °C), each cycle consisted of a denaturing step (1 min at 94 °C), annealing at 55 °C for 30 s and an extension step of 1 min at 72 °C. A final elongation step at 72 °C for 5 min preceded cooling at 4 °C. PCR products (16 μl) were loaded on a 5% non-denaturing acrylamide gel (*N*-acrylamide/*N*-methylenebisacrylamide, 37.5:1, Bio-Rad) and separated by electrophoresis (DSG200-02, C.B.S. Scientific) for 15 h at 8 mA. Gels were stained with SYBR green I (FMC Bioproducts) according to the manufacturer's instructions. RISA profiles were photographed and analysed using a Quantity One GelDoc2000 system (Bio-Rad) yielding data matrices which were used for principal component analysis (PCA). Those matrices took into account the presence or absence of bands and their normalized intensity. Variations in RISA profiles from different samples were assumed to reflect variation in the corresponding bacterial communities (Yannarell and Triplett, 2004).

2.4. Principal component analysis and classification

Principal component analysis (PCA) was used to evaluate similarities between RISA profiles. Similarities between samples were examined by hierarchical clustering analysis based on the Ward criterion performed in the subspace corresponding to 90% of the total variance. Major partitions were achieved by cutting dendrogram obtained in an optimum number and this clustering was consolidated by 10 iterations. PCA, clustering and plotting were conducted using SPAD software version 6.0 (Decisia).

2.5. Phylogenetical analysis of RISA bands

In order to identify predominant bacterial community members of the main specific RISA bands, a combined RISA/DGGE fingerprint method was used (Brown et al., 2005). This methodology makes it possible to relate a particular ITS length in RISA profiles to phylogenetic identification of main bacteria responsible for this fragment without cloning and sequencing several clones. The 16S-ITS rRNA region was amplified by PCR with primers GML5F (Goréguès et al., 2005) and FGPL132-38. PCR conditions were the same as for the RISA except for extension time which was 2.5 min. These PCR products were loaded on a 3.5% non-denaturing acrylamide gel and separated by size by electrophoresis for 7 h at 8.25 V cm^{-1}. Gels were stained and photographed as described above. Bands of interest were excised, transferred into 50 μl molecular biology grade water (Eppendorf, USA) and incubated for 12 h at 4 °C. One microliters of the supernatant was used as template for amplification of the partial 16S rRNA gene (568 pb) of the 16S-ITS rRNA fragments with 5′GC-clamped GML5F primer and primer 907RA (Goréguès et al., 2005). The differentiation of the amplicons on the basis of their sequence composition was performed by DGGE as described in Goréguès et al. (2005). Finally, dominant DGGE bands were excised from the DGGE gel and DNA eluted into 50 μl of DNA free water over night, reamplified with primer GM5F and 907RA and purified with Qiaquick PCR purification kit (Qiagen, USA) following the manufacturer's instructions. Each band was then sequenced by MWG Biotech (Germany).

2.6. Comparative analysis of 16S rRNA gene sequences

The sequences obtained in this study have been submitted to the DDBJ/EMBL/GenBank databases under the accession number DQ989499 to DQ989503. Sequences obtained were initially submitted to GenBank database of National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) using the Basic Local Alignment Search Tool algorithm to roughly determine their phylogenetic affiliation (Altschul et al., 1997). Sequences were checked by the CHECK_CHIMERA program of the Ribosomal Data Project (Maidak et al., 1999) to determine the presence of any PCR-amplified hybrid sequences, and then aligned with the same region of closest relative strains and uncultured clones using ClustalW facility (Thompson et al., 1994). Evolutionary distances were calculated from pair-wise sequence similarities with the Kimura 2-parameter model for nucleotide change using the DNADIST program available with the PHYLIP Package. A phylogenetic tree was constructed using the neighbour joining method. A bootstrap analysis with 1000 replicates was carried out to check the robustness of the tree which was visualized using the NJplot software (Perriere and Gouy, 1996).

3. Results

3.1. Effects of oil and N. diversicolor on bacterial community structures

After 45 days of incubation, BAL contamination induced major alterations in the RISA profiles (Fig. 1). Changes in banding patterns were mainly due to a decrease of the intensity of some previously existing bands and the appearance of new dominant bands (white arrows in Fig. 1). In the control inhabited microcosm (CTRL + Nd_{45}), the presence of *N. diversicolor* did not lead to visible changes in the RISA profiles. On the contrary, its presence in the contaminated sediments (OIL + Nd_{45}) both increased bands only existing in the oiled microcosm without polychaetes (OIL_{45}) and allowed the emergence of new ones (black arrows in Fig. 1).

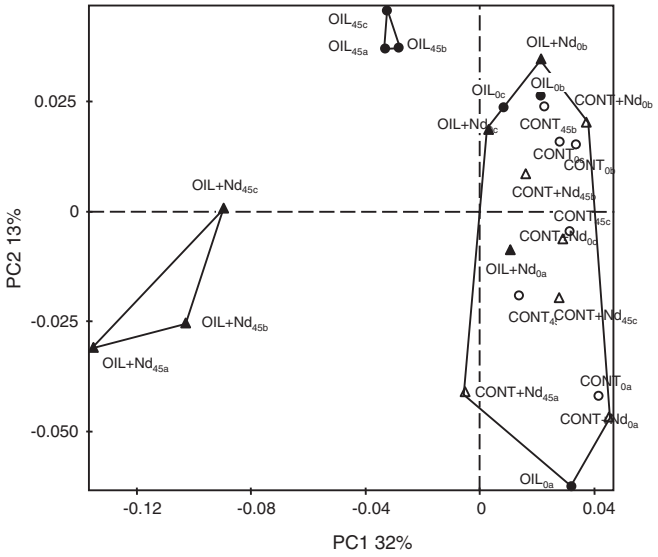

Fig. 1. Electrophoresis in 5% acrylamide gel of amplified bacterial intergenic transcribed spacer (ITS) region between 16S and 23S rRNA genes from DNA extracted from homogenised sediments coming from the four experimental microcosms at time zero ($CTRL_0$, $CTRL + Nd_0$, OIL_0, $OIL + Nd_0$) and after 45-days incubation ($CTRL_{45}$, $CTRL + Nd_{45}$, OIL_{45}, $OIL+Nd_{45}$). Arrows indicate emerging bands used for phylogenetic identification.

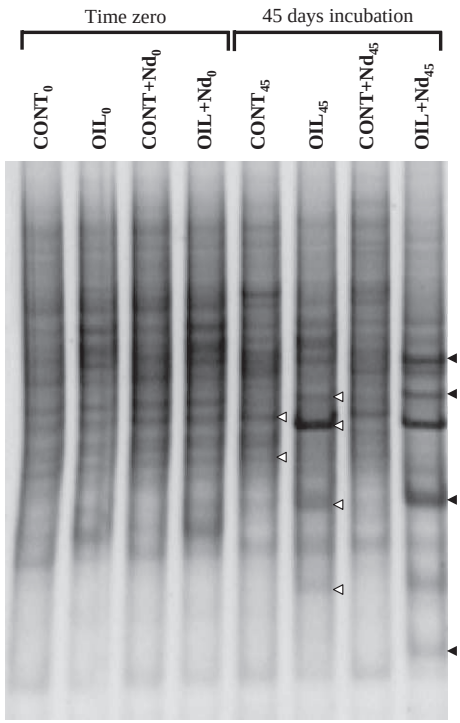

Fig. 2. PCA ordination ($PC1 \times PC2$) and hierarchical Ward clustering of triplicate RISA profiles (a, b and c) of bacterial communities in the four experimental microcosms at time zero ($CTRL_0$, $CTRL + Nd_0$, OIL_0, $OIL + Nd_0$) and after 45-days incubation ($CTRL_{45}$, $CTRL + Nd_{45}$, OIL_{45}, $OIL + Nd_{45}$).

PCA ordination and Ward clustering of the RISA profiles of bacterial communities of microcosms at the beginning of the experiment ($CTRL_0$, OIL_0, $CTRL + Nd_0$, $OIL + Nd_0$) and after 45 days of incubation ($CTRL_{45}$, OIL_{45}, $CTRL + Nd_{45}$, $OIL + Nd_{45}$) are shown in Fig. 2. The first principal component (PC1) of the PCA explained 32% of the total variance and the second principal component explained 13% of total variance. This PCA ordination demonstrated that all the microcosms at the beginning of the experiment but also the non-contaminated microcosms after 45 days of incubation ($CTRL_{45}$, $CTRL + Nd_{45}$) have a close bacterial community structure. Thus, the structure of the bacterial communities in non-contaminated sediments remained identical throughout the 45-days experiment even in the presence of *N. diversicolor*.

The addition of oil induced changes in the structure of the bacterial communities in the two types of contaminated sediments (with or without worms), but those changes appeared more pronounced for the sediments inhabited by *N. diversicolor* as shown along PC1 (Fig. 2). The presence of the polychaete in the oiled sediment ($OIL + Nd_{45}$) led to a specific bacterial community structure different (along PC1 and PC2) from that of the defaunated oiled sediment (OIL_{45}).

3.2. Phylogenetic affiliation of selected RISA bands

To characterize the main bacterial groups favoured by the presence of *N. diversicolor*, 13 partial 16S rRNA fragments obtained from the four specific RISA bands emerging or increasing in the OIL + Nd45 sediment were sequenced. A total of five different sequences (OSH1, OSH2, OSH3, OSH4 and OSH5) were found (length between 323 and 465 pb). A neighbour-joining tree was constructed with these sequences and related sequences from databases (Fig. 3). OSH1 showed 100% identity with *Alcanivorax* sp. OM-2 (Gamma Proteobacteria) while OSH2, OSH3 and OSH4 were only closely related to uncultured bacterium having no clear phylogenetic affiliation. Finally, OSH5 showed 99% and 98% identity with the Bacteroidetes *Formosa algae* clone SE60 and *Psychoserpens burtonensis* isolate S3-13, respectively.

4. Discussion

Ribosomal intergenic spacer analysis (RISA) is a molecular technique that utilizes the length heterogeneity of the intergenic transcribed spacer (ITS) region of bacterial rRNA operons to construct bacterial community "fingerprint" profiles. It was developed by Borneman and Triplett (1997) and was firstly applied to the study of microbial diversity in soils. This tool has been used successfully to assess community fingerprints, each band corresponding to at least one organism (Ranjard et al., 2000b; Kirk et al., 2004; Dorigo et al., 2005). This technique has been shown to be sensitive and robust for the detection of changes in bacterial communities following different kinds of disturbance (Robleto et al., 1998; Fisher and Triplett, 1999; Ranjard et al., 2000a; Selenska-Pobell et al., 2001).

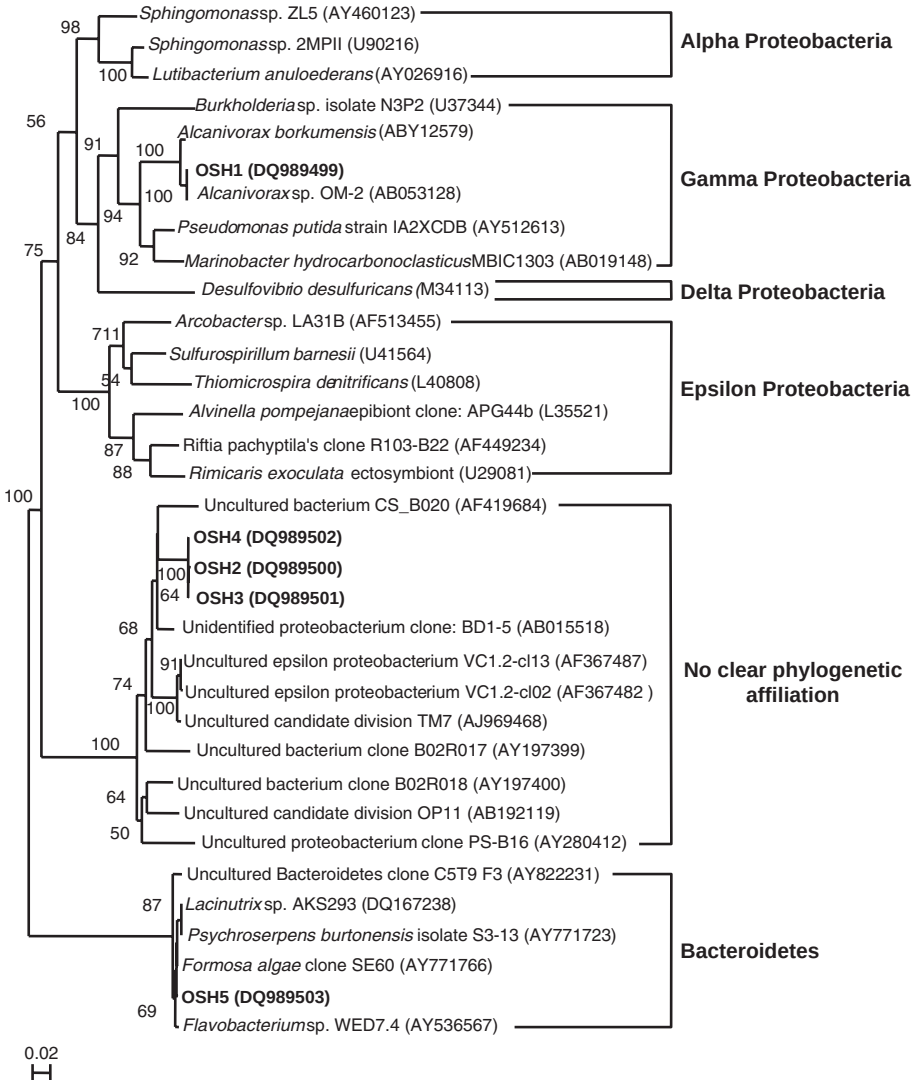

Fig. 3. Unrooted phylogenetic tree based on main partial 16S rRNA sequences obtained for the specific RISA bands from oiled sediments inhabited by *Nereis diversicolor*. Bootstrap values based on 1000 replicates each (for distance and parsimony) are shown for branches with more than 50% bootstrap values. The scale bar indicates 2% estimated sequence divergence.

In the present study, the oil contamination led to marked shifts in the structure of bacterial communities as attested by the multivariate analysis of the RISA profiles. This observation was not surprising since previous works have already reported changes in the composition and/or biomass of microbial communities in response to hydrocarbon contamination (Lindstrom et al., 1999; Katayama et al., 2003; Macnaughton et al., 2003; Maruyama et al., 2003; Röling et al., 2004; Yakimov et al., 2004; Yakimov et al., 2005; Yoshida et al., 2006). After contamination, hydrocarbonoclastic bacteria (HCB) usually dominate the contaminated system and Gram negative bacteria belonging to the *Proteobacteria* group seem to be particularly involved in natural cleansing processes (Macnaughton et al., 1999; Shi et al., 1999; Greene et al., 2000; Katayama et al., 2003).

However, our study provides information for the first time on the importance of bioturbation activity in selecting specific bacterial groups in oil contaminated sediments, this ''worm effect'' not being observed in the absence of oil. In the contaminated sediments inhabited by *N. diversicolor*, the bacterial communities exhibited specific RISA profiles which were different from those of macrofauna-defaunated contaminated sediments. These differences were mainly due to the increase of some bacterial groups and to the emergence of new ones. The presence of polychaetes favoured the development of specific bacterial groups, some of which are closely related to HCB like *Alcanivorax* sp. OM2 or *P. burtonensis*. Interestingly, the gamma Proteobacteria *Alcanivorax* sp. OM-2 is known to degrade *n*-alkanes whereas the *Flavobacteriaceae P. burtonensis* (Antarctic clone) uses aromatic hydrocarbons such as toluene, naphthalene, phenanthrene and anthracene as sole carbon sources (Yakimov et al., 1998). Moreover, *Alcanivorax* is a cosmopolitan alkane-degrading group of bacteria thought to play a critical role in the natural cleansing of oil-polluted marine

systems (Golyshin et al., 2002; Harayama et al., 2004). It is found in high abundance in oil-polluted waters and coastlines where it may comprise 80–90% of the oil-degrading microbial community (Kasai et al., 2001; Syutsubo et al., 2001; Harayama et al., 2004). The proportion of *Alcanivorax* was also shown to increase during laboratory enrichments with oil components (Yakimov et al., 1998; Harayama et al., 1999) and during field experiments involving the addition of nitrogen and phosphorus fertilizers to stimulate microbial degradation of oil (Kasai et al., 2002; Röling et al., 2002). Since all these bacteria are aerobic, the aerobic microbial community of the present reworked sediment may have been stimulated by the irrigation and the burrowing activities of *N. diversicolor* which can increase the penetration of oxygen into the sediment (Aller, 2001; Wenzhöfer and Glud, 2004). The feeding activity of the polychaete can also enhance hydrocarbon bioavailability to HCB by producing digestive solubilizers (Borneman and Triplett, 1997; Gilbert et al., 2001). Thus, both oxygenation and solubilization of oil hydrocarbons by *N. diversicolor* may have favoured the development of oxygenated zones and, subsequently, of HCB.

Our results may partly explain those of Christensen et al. (2002a) who showed, on the basis of several biodegradation indices, that the presence of *N. diversicolor* induced significant changes in the composition of oil. Undoubtedly, the selection of specific bacterial groups in oil contaminated sediments due to the presence and/or bioturbation activities of macrobenthic organisms requires further investigation. Primary factors affecting hydrocarbon bioremediation in marine sediments are the presence of HCB, optimum environmental conditions stimulating bacterial activity, the chemical composition of spilled petroleum and its relative toxicity and the bioavailability of hydrocarbons to HCB (Pelletier et al., 2004). The cultivation and introduction of indigenous polychaetes in contaminated marine environments may therefore constitute an original strategy for enhancing bioremediation processes.

On the other hand, the appearance of the new sequences OSH2, OSH3 and OSH4 likely to be related to a new division of uncultured bacteria (Fig. 3) suggests that the bacteria sharing these sequences have been inoculated in the sediments by *N. diversicolor*. Although this was beyond the scope of the present study, the full characterization (phylogeny and physiology) of the bacterial populations in bioturbated oil contaminated sediments also deserves further attention.

Acknowledgements

This work was supported by grants from CNRS-INSU (ATI 2001). Financial support from the French National Program ACI-Ecco (Ecodyn,"Indhyc") is also acknowledged. The authors thank Michael Paul for careful reading of the manuscript. This is Nereis Park contribution number 019.

References

Ahrens, M.J., Hertz, J., Lamoureux, E.M., Lopez, G.R., McElroy, A.E., Brownawell, B.J., 2001. The role of digestive surfactants in determining bioavailability of sediment-bound hydrophobic organic contaminants to 2 deposit-feeding polychaetes. Marine Ecology-Progress Series 212, 145–157.

Aller, R.C., 1994. Bioturbation and remineralization of sedimentary organic matter: effects of redox oscillation. Chemical Geology 114, 331–345.

Aller, R.C., 2001. Transport and reaction in the bioirrigated zone. In: Boudreau, B.P., Jørgensen, B.B. (Eds.), The Benthic Boundary Layer: Transport Processes and Biogeochemistry. Oxford University Press, Oxford, pp. 269–301.

Altschul, S., Madden, T., Schaffer, A., Zhang, J., Zhang, Z., Miller, W., Lipman, D., 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Research 25, 3389–3402.

Bauer, J.E., Kerr, R.P., Bautista, M.F., Decker, C.J., Capone, D.G., 1988. Stimulation of microbial activities and polycyclic aromatic hydrocarbon degradation in marine sediments inhabited by *Capitella capitata*. Marine Environmental Research 25, 63–84.

Bertrand, J.C., Bonin, P., Goutx, M., Mille, G., 1993. Biosurfactant production by marine microorganisms. Potential application to fighting hydrocarbon marine pollution. Journal of Marine Biotechnology 1, 125–129.

Bonin, P., Bertrand, J.C., 1999. Involvement of bioemulsifier in heptadecane uptake in *Pseudomonas nautica*. Chemosphere 38, 1157–1164.

Borneman, J., Triplett, E.W., 1997. Molecular microbial diversity in soils from eastern Amazonia: evidence for unusual microorganisms and microbial population shifts associated with deforestation. Applied and Environmental Microbiology 63, 2647–2653.

Brown, M.V., Schwalbach, M.S., Hewson, I., Fuhrman, J.A., 2005. Coupling 16S-ITS rDNA clone libraries and automated ribosomal intergenic spacer analysis to show marine microbial diversity: development and application to time series. Environmental Microbiology 7, 1466–1479.

Christensen, J.H., Hansen, A.B., Mortensen, J., Andersen, O., 2002a. A preliminary study on fate of oil in marine sediments inhabited by the polychaete *Nereis diversicolor*. In: Brebbia, C.A. (Ed.), Oil and Hydrocarbon Spills III: Modelling, Analysis and Control. Wessex Institute of Technology Press, Southampton, United Kingdom, pp. 289–300.

Christensen, M., Banta, G.T., Andersen, O., 2002b. Effects of the polychaetes *Nereis diversicolor* and *Arenicola marina* on the fate and distribution of pyrene in sediments. Marine Ecology-Progress Series 237, 159–172.

Chung, W.K., King, G.M., 1999. Biogeochemical transformations and potential polyaromatic hydrocarbon degradation in macrofaunal burrow sediments. Aquatic Microbial Ecology 19, 285–295.

Chung, W.K., King, G.M., 2001. Isolation, characterization, and polyaromatic hydrocarbon degradation potential of aerobic bacteria from marine macrofaunal burrow sediments and description of *Lutibacterium anuloederans* gen. nov., sp. nov., and *Cycloclasticus spirillensus* sp. nov. Applied and Environmental Microbiology 67, 5585–5592.

Dobbs, F.C., Guckert, J.B., 1988. Microbial food resources of the macrofaunal-deposit feeder Ptychodera bahamensis (Hemichordata: Enteropneusta). Marine Ecology-Progress Series 45, 127–136.

Dorigo, U., Volatier, L., Humbert, J.-F., 2005. Molecular approaches to the assessment of biodiversity in aquatic microbial communities. Water Research 39, 2207–2218.

Findlay, R.H., Trexler, M.B., White, D.C., 1990. Response of a benthic microbial community to biotic disturbance. Marine Ecology-Progress Series 62, 135–148.

Fisher, M.F., Triplett, E.W., 1999. Automated approach for ribosomal intergenic spacer analysis of microbial diversity and its application to

freshwater bacterial communities. Applied and Environmental Microbiology 65, 4630–4636.
Gardner, W.S., Lee, R.F., Tenore, K.R., Smith, L.W., 1979. Degradation of selected polycyclic aromatic hydrocarbons in coastal sediments: importance of microbes and polychaete worms. Water, Air, and Soil Pollution 11, 339–347.
Gilbert, F., Stora, G., Desrosiers, G., Deflandre, B., Bertrand, J.-C., Poggiale, J.-C., Gagne, J.-P., 2001. Alteration and release of aliphatic compounds by the polychaete *Nereis virens* (Sars) experimentally fed with hydrocarbons. Journal of Experimental Marine Biology and Ecology 256, 199–213.
Golyshin, P.N., Chernikova, T.N., Abraham, W.R., Lunsdorf, H., Timmis, K.N., Yakimov, M.M., 2002. Oleiphilaceae fam nov., to include *Oleiphilus messinensis* gen. nov., sp. nov., a novel marine bacterium that obligately utilizes hydrocarbons. International Journal of Systematic and Evolutionary Microbiology 52, 901–911.
Goñi-Urriza, M., de Montaudouin, X., Guyoneaud, R., Bachelet, G., de Wit, R., 1999. Effect of macrofaunal bioturbation on bacterial distribution in marine sandy sediments, with special reference to sulphur-oxidising bacteria. Journal of Sea Research 41, 269–279.
Goréguès, C.M., Michotey, V.D., Bonin, P.C., 2005. Molecular, biochemical, and physiological approaches for understanding the ecology of denitrification. Microbial Ecology 49, 198–208.
Granberg, M.E., Hansen, R., Selck, H., 2005. Relative importance of macrofaunal burrows for the microbial mineralization of pyrene in marine sediments: impact of macrofaunal species and organic matter quality. Marine Ecology-Progress Series 288, 59–74.
Greene, E.A., Kay, J.G., Jaber, K., Stehmeier, L.G., Voordouw, G., 2000. Composition of soil microbial communities enriched on a mixture of aromatic hydrocarbons. Applied and Environmental Microbiology 66, 5282–5289.
Grossi, V., Massias, D., Stora, G., Bertrand, J.-C., 2002. Burial, exportation and degradation of acyclic petroleum hydrocarbons following a simulated oil spill in bioturbated Mediterranean coastal sediments. Chemosphere 48, 947–954.
Grossi, V., Cuny, P., Caradec, S., Nérini, D., Pancost, R., Gilbert, F., 2006. Impact of feeding by *Arenicola marina* (L.) and ageing of faecal material on fatty acid distribution and bacterial community structure in marine sediments: an experimental approach. Journal of Experimental Marine Biology and Ecology 336, 54–64.
Grossmann, S., Reichardt, W., 1991. Impact of *Arenicola marina* on bacteria in intertidal sediments. Marine Ecology-Progress Series 71, 85–96.
Harayama, S., Kishira, H., Kasai, Y., Syutsubo, K., 1999. Petroleum biodegradation in marine environments. Journal of Molecular Microbiology and Biotechnology 1, 63–70.
Harayama, S., Kasai, Y., Hara, A., 2004. Microbial communities in oil-contaminated seawater. Current Opinion in Biotechnology 15, 205–214.
Head, I.M., Swannell, R.P., 1999. Bioremediation of petroleum hydrocarbon contaminants in marine habitats. Current Opinion in Biotechnology 10, 234–239.
Kasai, Y., Kishira, H., Syutsubo, K., Harayama, S., 2001. Molecular detection of marine bacterial populations on beaches contaminated by the Nakhodka tanker oil-spill accident. Environmental Microbiology 3, 246–255.
Kasai, Y., Kishira, H., Sasaki, T., Syutsubo, K., Watanabe, K., Harayama, S., 2002. Predominant growth of *Alcanivorax* strains in oil-contaminated and nutrient-supplemented sea water. Environmental Microbiology 4, 141–147.
Katayama, Y., Oura, T., Iizuka, M., Orita, I., Cho, K.J., Chung, I.Y., Okada, M., 2003. Effects of spilled oil on microbial communities in a tidal flat. Marine Pollution Bulletin 47, 85–90.
Kirk, J.L., Beaudette, L.A., Hart, M., Moutoglis, P., Klironomos, J.N., Lee, H., Trevors, J.T., 2004. Methods of studying soil microbial diversity. Journal of Microbiological Methods 58, 169–188.
Lindstrom, J.E., Barry, R.P., Braddock, J.F., 1999. Long-term effects on microbial communities after a subarctic oil spill. Soil Biology and Biochemistry 31, 1677–1689.
Macnaughton, S.J., Stephen, J.R., Venosa, A.D., Davis, G.A., Chang, Y.J., White, D.C., 1999. Microbial population changes during bioremediation of an experimental oil spill. Applied and Environmental Microbiology 65, 3566–3574.
Macnaughton, S.J., Swannell, R., Daniel, F., Bristow, L., 2003. Biodegradation of dispersed forties crude and Alaskan North slope oils in microcosms under simulated marine conditions. Spill Science & Technology Bulletin 8, 179–186.
Maidak, B., Cole, J., Parker Jr., C., Garrity, G., Larsen, N., Li, B., Lilburn, T., McCaughey, M., Olsen, G., Overbeek, R., Pramanik, S., Schmidt, T., Tiedje, J., Woese, C., 1999. A new version of the RDP (Ribosomal Database Project). Nucleic Acids Research 27, 171–173.
Maruyama, A., Ishiwata, H., Kitamura, K., Sunamura, M., Fujita, T., Matsuo, M., Higashihara, T., 2003. Dynamics of microbial populations and strong selection for *Cycloclasticus pugetii* following the *Nakhodka* oil spill. Microbial Ecology 46, 442–453.
McElroy, A.E., Farrington, J.W., Teal, J.M., 1990. Influence of mode of exposure and the presence of a tubiculous polychaete on the fate of benz[a]anthracene in the benthos. Environmental Science and Technology 24, 1648–1655.
Myers, A., 1977. Sediment processing in a marine subtidal sandy bottom: I. Physical aspects. Journal of Marine Research 35, 609–632.
Normand, P., Ponsonnet, C., Nesme, X., Neyra, M., Simonet, P., 1996. ITS analysis of prokaryotes. In: Akkermans, D.L., van Elsas, J.D., de Bruijn, E.I. (Eds.), Molecular microbial ecology manual. Kluwer Academic Publishers., Amsterdam, The Netherlands, pp. 1–12.
Papaspyrou, S., Gregersen, T., Cox, R.P., Thessalou-Legaki, M., Kristensen, E., 2004. Sediment properties and bacterial community in burrows of the ghost shrimp *Pestarella tyrrhena* (Decapoda: Thalassinidea). Aquatic Microbial Ecology 38, 181–190.
Papaspyrou, S., Gregersen, T., Kristensen, E., Christensen, B., Cox, R.P., 2006. Microbial reaction rates and bacterial communities in sediment surrounding burrows of two nereidid polychaetes (*Nereis diversicolor* and *N. virens*). Marine Biology 148, 541–550.
Pelletier, E., Delille, D., Delille, B., 2004. Crude oil bioremediation in sub-Antarctic intertidal sediments: chemistry and toxicity of oiled residues. Marine Environmental Research 57, 311–327.
Penry, D.L., Weston, D.P., 1998. Digestive determinants of benzo[a]pyrene and phenanthrene bioaccumulation by a deposit-feeding polychaete. Environmental Toxicology and Chemistry 17, 2254–2265.
Perriere, G., Gouy, M., 1996. www-query: an on-line retrieval system for biological sequence banks. Biochimie 78, 364–369.
Plante, C.J., Wilde, S.B., 2004. Biotic disturbance, recolonization, and early succession of bacterial assemblages in intertidal sediments. Microbial Ecology 48, 154–166.
Ranjard, L., Nazaret, S., Goubière, F., Thioulouse, J., Linet, P., Richaume, A., 2000a. A soil microscale study to reveal the heterogeneity of Hg(II) impact on indigenous bacteria by quantification of adapted phenotypes and analysis of community DNA fingerprints. FEMS Microbiology Ecology 31, 107–115.
Ranjard, L., Poly, F., Nazaret, S., 2000b. Monitoring complex bacterial communities using culture-independent molecular techniques: application to soil environment. Research in Microbiology 151, 167–177.
Robleto, E.A., Borneman, J., Triplett, E.W., 1998. Effects of bacterial antibiotic production on rhizosphere microbial communities from a culture-independent perspective. Applied and Environmental Microbiology 64, 5020–5022.
Röling, W.F.M., Milner, M.G., Jones, D.M., Lee, K., Daniel, F., Swannell, R.J.P., Head, I.M., 2002. Robust hydrocarbon degradation and dynamics of bacterial communities during nutrient-enhanced oil spill bioremediation. Applied and Environmental Microbiology 68, 5537–5548.
Röling, W.F.M., Milner, M.G., Jones, D.M., Fratepietro, F., Swannell, R.P.J., Daniel, F., Head, I.M., 2004. Bacterial community dynamics and hydrocarbon degradation during a field-scale evaluation of bioremediation on a mudflat beach contaminated with buried oil. Applied and Environmental Microbiology 70, 2603–2613.

Roose-Amsaleg, C.L., Garnier-Sillam, E., Harry, M., 2001. Extraction and purification of microbial DNA from soil and sediments samples. Applied Soil Ecology 18, 47–60.

Selenska-Pobell, S., Kampf, G., Flemming, K., Radeva, G., Satchanska, G., 2001. Bacterial diversity in soil samples from two uranium waste piles as determined by rep-APD, RISA and 16S rDNA retrieval. Antonie van Leeuwenhoek International Journal of General and Molecular Microbiology 79, 149–161.

Shi, Y., Zwolinski, M.D., Schreiber, M.E., Bahr, J.M., Sewell, G.W., Hickey, W.J., 1999. Molecular analysis of microbial community structures in pristine and contamined aquifers : field and laboratory microcosm experiments. Applied and Environmental Microbiology 65, 2143–2150.

Syutsubo, K., Kishira, H., Harayama, S., 2001. Development of specific oligonucleotide probes for the identification and *in situ* detection of hydrocarbon-degrading *Alcanivorax* strains. Environmental Microbiology 3, 371–379.

Thompson, J., Higgins, D., Gibson, T., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research 22, 4673–4680.

Tsai, Y.-L., Olson, B.H., 1991. Rapid method for direct extraction of DNA from soil and sediments. Applied and Environmental Microbiology 57, 1070–1074.

Wenzhöfer, F., Glud, R., 2004. Small-scale spatial and temporal variability in coastal benthic O_2 dynamics: Effects of fauna activity. Limnology and Oceanography 49, 1471–1481.

Weston, D.P., Mayer, L.M., 1998. *In vitro* digestive fluid extraction as a measure of the bioavailability of sediment-associated polycyclic aromatic hydrocarbons: sources of variation and implications for partitioning models. Environmental Toxicology and Chemistry 17, 820–829.

Wilde, S.B., Plante, C.J., 2002. Spatial heterogeneity of bacterial assemblages in marine sediments: the influence of deposit feeding by *Balanoglossus aurantiacus*. Estuarine, Coastal and Shelf Science 55, 97–107.

Yakimov, M., Golyshin, P., Lang, S., Moore, E., Abraham, W., Lunsdorf, H., Timmis, K., 1998. *Alcanivorax borkumensis* gen. nov., sp. nov., a new, hydrocarbon-degrading and surfactant-producing marine bacterium.. International Journal of Systematic Bacteriology 48, 339–348.

Yakimov, M.M., Gentile, G., Bruni, V., Cappello, S., D'Auria, G., Golyshin, P.N., Giuliano, L., 2004. Crude oil-induced structural shift of coastal bacterial communities of rod bay (Terra Nova Bay, Ross Sea, Antarctica) and characterization of cultured cold-adapted hydrocarbonoclastic bacteria. FEMS Microbiology Ecology 49, 419–432.

Yakimov, M.M., Denaro, R., Genovese, M., Cappello, S., D'Auria, G., Chernikova, T.N., Timmis, K.N., Golyshin, P.N., Giluliano, L., 2005. Natural microbial diversity in superficial sediments of Milazzo Harbor (Sicily) and community successions during microcosm enrichment with various hydrocarbons. Environmental Microbiology 7, 1426–1441.

Yannarell, A.C., Triplett, E.W., 2004. Within- and between-lake variability in the composition of bacterioplankton communities: investigations using multiple spatial scales. Applied and Environmental Microbiology 70, 214–223.

Yoshida, A., Nomura, H., Toyoda, K., Nishino, T., Seo, Y., Yamada, M., Nishimura, M., Wada, M., Okamoto, K., Shibata, A., 2006. Microbial responses using denaturing gradient gel electrophoresis to oil and chemical dispersant in enclosed ecosystems. Marine Pollution Bulletin 52, 89–95.